LXXXII. NOTES ON THE DEVELOPMENT OF *BACILLUS PESTIS* IN BUGS (*CIMEX LECTULARIUS*) AND THEIR POWER TO CONVEY INFECTION.

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(With Plates XXXVII and XXXVIII and 1 Text-figure.)

Introduction.

Verjbitzki (1904) was quite successful in his attempts to infect guinea-pigs by the bites of bugs (*Cimex lectularius*) which had previously been allowed to feed on animals that were dying of plague.

He found that allowing the insects to feed on the ear was more effective than letting them bite the leg; while, apart from situation, the virulence of the strain which killed the animals on which the bugs were fed was the crucial factor.

When the virulence was low there were no deaths, but, with a higher virulence, deaths occurred and the percentage of animals dying increased with the virulence of the strain of *B. pestis* used. Bugs that had been starved for longer or shorter periods were used, and it was discovered that the longer the period of starvation prior to the infected meal, the longer the period that the bacilli could be recovered from the bug. The longest time after infection that *B. pestis* could be recovered by culture was eight days, but the longest period after infection that the disease could be conveyed by bite was five days. The smallest number of infected bugs used successfully to convey infection was three. The same batch of infected bugs was able to transmit the disease to two batches of animals on successive days, but failed on the third occasion.

Nuttall (1899—1900) tried on four occasions to convey the disease to mice by means of infected bugs, but failed on each occasion. He was able to recover *B. pestis* from the body of bugs five days after the infected meal.

Jordansky and Klodnitsky (1908) found that attempts to convey plague by allowing bugs to feed on a mouse sick of the disease, and before they were satisfied removing them to a healthy mouse, failed. They noted that the number of bacilli in the bug's stomach increased from the third to the sixth day after the infected meal. On the tenth day involution forms appeared, and subsequently, although the bacilli disappeared from view, they were recoverable by culture after 35 days.

Jordansky and Klodnitsky (1910) stated that, of 13 bugs fed on a pest infected mouse, two survived for 83 days and were then fed on a healthy animal. Five days later *B. pestis* was demonstrated in the bugs both by microscope and culture.

The following notes are the outcome of some rather desultory work that was performed as time and opportunity permitted during the course of a more systematic research concerning the transference of pest by fleas.

Methods.

Bugs (*Cimex lectularius*) were infected by allowing them to feed on mice that were in the comatose condition that immediately precedes death from plague. In attempting to reconvey the disease to mice by allowing the bugs to feed on them, it was found that active healthy mice eat the bugs unless some provision is made to afford the insects cover from their attacks. Mice can of course be bound, but they are restless in this condition and it was especially desired to allow the bugs a chance of undisturbed feeding while the mice slept. The usual points of attack are apparently the ears, tail or feet. A simple method of overcoming the difficulty is to bore a one inch hole in a wooden block some three or more inches long and then to make a number of saw cuts in the block so as to penetrate into the hole. A suitable tube can be arranged by cutting the bases off two solid wooden postal blocks, such as are used for despatching specimen tubes, and then nailing them together at right angles (fig. 1).

Unfortunately this plan was not devised until after such infection experiments as the time at my disposal allowed for, had been carried out. The method was found to be successful, however, in so far as by its adoption it was found possible for bugs to be kept with active mice in a cage suitable for infection experiments, and to feed freely upon them.

The effect of infected blood upon the bugs.

Bugs in their first instar as well as older larvae, nymphs and adults, were infected. The infected blood was the apparent cause of considerable mortality among the insects; especially was this the case with the first instar larvae[1].

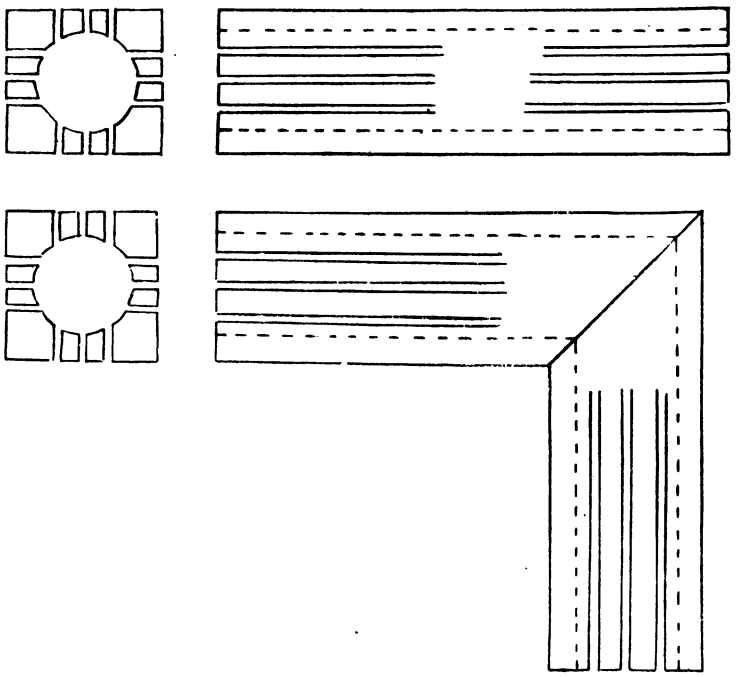

Fig. 1.

Twenty young bugs that had hatched a week or two previously were allowed to make their first meal on an infected mouse. Most of them fed to their full extent. After feeding they were placed in an entomological glass-bottomed card box and kept in a dry cupboard at 60—69° F. Two, which appeared to be injured, died the day following their meal,

[1] To avoid any confusion I must explain that the conditions under which bugs are kept after a full meal are important, even if the food be normal human blood. Placed at the bottom of a glass tube in a cool, moist situation, there was considerable mortality in my experience, whereas control insects kept in card boxes in a dryer and warmer situation showed no deaths. Young (first instar) bugs are more delicate in this respect than older larvae, nymphs or adults.

and an examination of the blood in their stomachs showed that it was heavily infected with *B. pestis.*

Three moulted four days after feeding, and then died; thirteen which had not moulted after seven days, were found lying at the bottom of the box, either recently dead or paralyzed. The two remaining did not moult, and were still active on the eighth day, but were found in the same paralyzed condition as the others on the tenth day.

As regards external appearances, the only unusual feature noticeable in these young bugs and the older ones which died in a similar paralyzed condition, after full meals of infected blood, was their failure to reduce their bulk, a process which is normally noticeable in healthy specimens within a few hours after a full meal. In this respect their symptoms are similar to those which accompany the death of normally fed bugs which have been kept in too cool and moist a situation after a full meal.

Smears of the stomach contents of infected bugs made at various periods after the infected meal show the following appearances. In the majority of the cases the specimens were paralyzed or dying at the time they were dissected. In only a few instances were healthy or definitely dead insects used, but the actual condition of the insects at the time of making the preparation made no apparent difference.

STOMACH CONTENTS TREATED AS BLOOD SMEARS.

(*Stains used—Leishman or Carbol-Thionin.*)

Period and temperature between infecting meal and death of bug — Remarks

1 day at about 65° F.

Some large bipolar staining bacilli present, but the infection is chiefly of the "Yeast" like forms photographed by Dr Rowland (1914) in his recent paper on "The Morphology of the Plague Bacillus," figs. 16 and 17. The organisms are generally scattered in the smear—there is no clustering. Phagocytes intact and numerous. (1 specimen.)

3 days at about 80° F.

A very dense growth of the small culture form (see figs. 1 and 2 in Rowland's paper). No "Yeast" forms, a few large forms and many smaller ones show bipolar staining; a tendency to cluster and clump is noticeable. There are but few phagocytes visible. (2 specimens.)

3 days at 60° F.

Growth much less dense than in the last—mostly consisting of the "Yeast" form of the small culture form; a few large bacilli, which show bipolar staining. No clustering. A slight tendency to form chains, but this does not progress beyond threes and fours of the slender type, phagocytes not numerous. (1 specimen.)

A second specimen shows a denser growth with the phagocytes more numerous. A third agrees with the second, but has a rather more varied assortment of form, and fewer of the "Yeast" form.

5 days at 65° F.

This differs but little from the 3-day specimens, but there is no trace of any "chain" tendency.

5 days at 32° F.

Bacteria few and scattered; all large form, often short and mostly showing bipolar staining; blood appears unaltered in structure. (1 specimen.)

5 days at 80° F.

Very numerous "Yeast" and short, broad bipolar staining types; a few very long and broad and a few long and narrow, both bipolar staining. A very noticeable feature are the clumps of unstainable material, presumably of autolyzed blood and unstainable bacteria. No clustering of the staining forms. (1 specimen.)

7 days at 65° F.

Bacteria very numerous, scattered, of the "Yeast" type, with a certain admixture of large, short bipolar staining forms, and in one specimen a small proportion of the slender bipolar forms also. Phagocytes are present, but the smears are too poor to allow of any decision as to their condition. (4 specimens.)

8 days at 60—65° F.

Very numerous, the greater portion of the "Yeast" form; a few pairs of the slender forms set end to end, but no actual chains. There is also a sprinkling of the large bipolar staining forms, mostly rather stumpy. Same tendency to clustering is noticeable, but no actual clumps. Phagocytes are present. (4 specimens.)

10 days at 60—65° F.

Large clumps composed of autolyzed blood, and masses of the bodies of bacteria which do not stain are present. Mixed with them in the clumps, and also generally distributed throughout the smear, are numerous well-stained forms, mostly of the "Yeast" type. There is also a sprinkling of large bipolar staining bacilli, for the most part rather short. The general appearance reminds one of some of the phases found in fleas, with the modification that recognizable phagocytes are present after ten days. (1 specimen.)

Period and temperature between infecting meal and death of bug — Remarks

10 days at 60—65° F.

A second specimen shows neither the clumps of autolyzed blood nor the non-staining bacteria, but only a general distribution of numerous "Yeast" forms and some other types. The general appearance is very different from the last. The blood has apparently been but little altered—red blood cells and phagocytes being present in numbers. (1 specimen.)

15 days at 60° F.

This bug had only had a small meal. The stomach contents mostly consist of unstainable material—clusters of the non-staining bacteria being mixed with the autolyzed blood. A very few and widely scattered stained forms are present and some of these show bipolar staining. No phagocytes discernible. (1 specimen.)

15 days at 65—70° F.

Various small faintly staining forms are present, mostly short; in many cases showing bipolar staining. A few pairs visible; recognizable phagocytes rare. (1 specimen.)

21 days at 28—30° F.

Large bipolar forms, many dividing and a few short chains. The bacilli few and scattered; the blood apparently unaltered structurally. (1 specimen.)

21 days at 65° F.

Numerous but scattered, for the most part small, inclining to the "Yeast" form, but some bipolars of small size are present. Phagocytes still easily recognizable.

60 days at 80° F.

The smear shows masses of small coccus-shaped bodies embedded in the autolyzed matter of which the smear is made. These bodies are not very sharp in outline and are usually unstained and of a dull greenish hue. I have, however, come across one or two small groups of similar bodies which are faintly stained. When compared with definite pest smears, one hesitates, in spite of the wide variation of *B. pestis*, to say that these minute and indistinct bodies are pest bacteria, nevertheless I suspect this to be the case and look upon them as the dead or degenerate remains of a starved culture. (1 specimen.) In this connection note the following example.

A bug that had fed on a mouse dying of pest was then fed at intervals of 3, 7, 18 and 19 days on a normal rat, which did not contract the disease. The bug died on the 40th day after the infected meals. The bacteria present in the stomach smear are very numerous and much smaller than those in the other smears, in some cases they are as minute as the bodies in the last specimen referred to above. In shape they are mostly of

either the "Culture" or "Yeast" forms, but others still, if unusually small, are of the slender type arranged in twos and threes. A few of the normal blood forms showing bipolar staining are present—these also being very small in comparison with similar forms on other smears. All of these types stain more heavily than usual. Red blood cells and phagocytes are numerous and easily discernible, but the former appear in poor condition —being much distorted in shape.

It will be seen that the characteristic form of *B. pestis* in these smears is that which Rowland (1914) has termed "Yeast" like forms and of which he shows two photographs, figs. 16 and 17. Speaking from memory, I do not recollect to have found this form in preparations of the fleas' stomachs, certainly it was never a distinctive feature as it is in the bug. As was to be expected, the number of bacteria present is to a considerable extent dependent on the period and temperature between the infected meal and the death of the bug. But, apart from this general result, to which there are some exceptions, it does not seem possible to draw any very definite conclusion as to the relationship between temperature or period and the type of *B. pestis* present or the development of the growth. There is, however, as in the flea, a definite association between the presence of unstainable bacteria and dark autolyzed material; also a suggestion that agglomerated growth may be associated with the destruction of the blood cells.

A clear, colourless fluid present in the rectum of many of the first instar bugs which were killed or paralyzed as the result of the infected meal, was squeezed out prior to the rupture of the crop. This showed no trace of *B. pestis* on microscopic examination.

Sections.

The bugs, after fixation, were embedded in clove oil celloidin and paraffin (an adaptation of Entz's method, *Arch. Protistenk*, xv. 1909, p. 98), and then stained with Haematoxylin and Eosin for tissues and blood, subsequently with Carbol-Thionin for the bacilli.

SECTIONS OF PEST INFECTED BUGS.

(*Stained Haematoxylin, Eosin and Carbol-thionin.*)

Period and temperature between infecting meal and death of bug — Remarks

1 day at 60—65° F. (Pl. XXXVII, fig. 1.)

Transverse about the middle of crop (bug in first instar), × 180. The infection is not generally diffused; it appears to be of a stratified nature, but with many small detached colonies. The dorsal area is decidedly freer than the ventral, suggesting a gravitational effect which is not, however, consistently borne out, as there is one large wave of infected blood in the dorsal area which comes in contact with the upper wall of the crop. There is a very definite association of the phagocytes in the blood with the bacteria, quite apart from any gravitational process. The growth of the bacilli must have been very rapid to have progressed to such an extent in 24 hours. Many of the sections show curiously sharp lines of demarcation between the masses of infected and uninfected blood. A belt of normal blood of remarkably even thickness, coming between a less regular one, carrying a mass of bacteria and phagocytes, and the wall of the crop may be observed. The division is as sharp as if a line had been drawn.

With a 1/12″ oil immersion lens (Pl. XXXVII, fig. 2) the bacilli are seen to be all of the culture form and the higher magnification also reveals numerous darkly staining bodies of varying sizes, larger than a bacterium and smaller than a red blood cell. From the almost invariable association of these particles with infected areas where phagocytes are present, it would seem definite that they are not normal to the blood or fluids of the bug's stomach. Appearances suggest that they are fragments of the nuclei of dead phagocytes.

2 days at 60—65° F. (Plate XXXVIII.)

Longitudinal vertical section of adult bug. The crop much distended with blood, × 180. The central area shows very numerous patches of a dirty brown colour which contrast sharply with the eosin stained blood. These patches vary greatly in both size and shape; towards the anterior end of the crop is a very large patch of this description. The central area of this patch is fragmentary and loose in character, but towards its periphery it becomes very dense and within this dense belt large numbers of phagocytes are massed together. At its outer margin there is a more or less broken brightly staining fringe.

With the 1/12″ oil immersion lens (Pl. XXXVII, fig. 3) the dark patches above referred to are all found to be very similar in character, consisting of masses of small unstainable bodies which by their number and agreement in shape I take to be bacteria which do not take the stain, and fragments of varied, usually large size and irregular shaped (presumably autolyzed) blood cells or fragments of them. At the periphery of these masses there is a more or less broken fringe of stained bacilli. There

are also occasional, small patches of stained bacilli among the red blood cells quite apart from the masses of autolyzed material. The phagocytes are almost invariably confined within the autolyzed areas—the larger and presumably less recently developed being disproportionally favoured in this respect; in many instances they appear distorted and fragmentary. A further difference between the very large and the smaller patches is that the former alone show a degenerating central area. The same phenomena, mentioned in the account of the first specimen, of sharp lines of demarcation between infected and uninfected areas is apparent in this specimen as well. The appearance suggests that masses of bacteria had developed against the wall of the crop until they had attained some cohesion and then becoming shifted, uninfected blood had flowed in between them and the walls of the crop. In this specimen blood is present in the oesophagus from its entrance into the crop forward to the posterior end of the pump. A few stained bacteria are to be observed in the tube in front of its passage through the brain.

5 days at 60—65° F.

Longitudinal vertical section of adult bug, × 180. The development in this specimen differs very greatly from that in the last. The red cells are very distinct, but few if any phagocytes are present in the crop, while in place of the stratified appearance after the one-day infection or the large autolyzed clumps in the last specimen, there is a much more even distribution of small patches of stained bacilli with slender ramifications. The autolyzed material consists of generally distributed specks. The slender portions of the stomach behind the crop and the gut, however, which were more or less empty in the other specimens, are in this one distended with partially digested material. Large numbers of red blood cells, more or less perfect in outline, are present in both tubes, a few only of which have taken the stain, the others are mere outlines of the same tint as the remainder of the autolyzed material.

With a 1/12″ immersion lens it is seen that this unstained material consists of what are probably fragments of blood cells and masses of unstained bacteria, with a few well-stained examples among them. In smaller areas the material present consists of masses of stained bacilli with a smaller admixture of unstainable ones. A few well-stained but distorted or fragmentary phagocytes are also visible in the lower stomach.

The difference between this and the last specimen in fact amounts to the segregation of the phagocytes and larger masses of autolyzed material to the lower portion of the stomach and gut on the one hand and their general distribution in the central area of the crop on the other.

It seems not impossible that such a result might follow the withdrawal of blood from the crop to the lower stomach, as no doubt the central area would respond to suction more readily than the outer layers, but the completeness of the segregation of the leucocytes arrived at in this specimen seems hardly credible by these means alone.

This specimen also shows blood in the oesophagus and the posterior end of the pumping chamber. There are a few bacilli to be found free in this

Period and temperature between infecting meal and death of bug — Remarks

blood, while in the folds and puckers formed by the walls of the oesophagus at its junction with the pump, dense clusters are to be seen from which small detached groups of bacilli are passing out through the narrow entrance into the pump.

8 days at 60—65° F.

Longitudinal vertical section of a first instar bug; ×180. The development in this specimen consists chiefly of a large infected area within the crop, in addition to which there are some streaks ramifying from this centre with small detached patches. The lower, tubular portion of the stomach contains large masses of partially digested blood, which has not stained, while the contents of the crop at the posterior angle look dense and are of a yellowish colour. The leucocytes are as usual almost all crowded into the infected area.

With the 1/12″ immersion lens the bacteria are seen to be clustered within the central area and other infected patches, and to stain vividly. The same dark staining spots previously described in the account of the first specimen (1 day) are present within the large infected area. Their appearance in this specimen bears out the previously made suggestion that they are fragments of the nuclei of the leucocytes. The yellowish area at the extremity of the crop seems to consist of clotted blood; it is very dense and shows no structure, while the bacilli are thickly and evenly distributed, giving the patch a granular appearance.

The autolyzed material in the lower portion of the stomach is seen to consist of more or less digested blood, mixed with large clumps of unstained bacteria.

A second specimen, *cut horizontally*, shows a much weaker infection in the crop, but the posterior portion of the stomach, or a fold of the intestine is closely packed with a dense mass of vividly stained bacilli, the unstained autolyzed material being confined to a small central portion of the tube in this section. This is by far the densest and, presumably, the most rapid growth I have seen in the bug and approaches more nearly to that which occurs in the laked blood within the stomach of the flea than any of the specimens cut as sections have yet shown.

A third specimen, *cut transversely*, agrees pretty closely with the 1-day specimen as regards the development within the crop, but the tubular portion of the alimentary system is seen to be well filled with unstained material. With the 1/12″ objective this latter is seen to consist as usual of more or less broken down blood cells and dense masses of unstained bacteria.

A fourth specimen, *cut in longitudinal vertical direction*, agrees closely with the last in respect of the development of the infecting growth. It also shows blood to be present in the pumping chamber, which is heavily infected in places.

34 days at 60—65° F.

Longitudinal vertical section of a nymph. All the blood in the crop has been broken down, and there are no signs of bacilli in it. The autolyzed material in the tubular portion of the alimentary system contains numbers of small specks which differ considerably from the appearance of the unstained bacteria which give a granular appearance in the definitely infected bugs. There is, of course, a possibility that they may be connected with a previous growth of bacteria, but I have seen very similar objects among the autolyzed material in the intestines of normally fed bugs, and am of opinion that this insect was not infected at the time of death.

74 days at 60—65° F.

Longitudinal vertical section of adult. There is no definite evidence that this specimen was infected when it died. The autolyzed contents of the alimentary canal show numbers of the small unstained bodies which are possibly the remnants of bacteria, but beyond the fact that their size and distribution are more favourable to this view than in the last (34-day specimen) it is not possible to go with safety.

The sections do not give such clear details of the character of the individual bacilli as do smears, for the following reasons, among others: (1) Dehydration causes the bacteria to shrink whereas, in a smear, shrinkage is prevented; (2) The sections are rather thick (about 5 or 6 μ); (3) The bacilli are thickly clustered together; (4) The staining, Haematoxylin and Thionin, is not very distinctive.

In all but the last two specimens dealt with (34 days and 74 days) the presence of unstained bacteria among the autolyzed material in the gut can be definitely determined, but with regard to these last there is considerable doubt, as the small dark bodies present approach more nearly to those seen among the detritus in the intestines of normally fed specimens of *Cimex lectularius* and *hirundinis*.

The points of most general interest appear to be the very definite association between the leucocytes and the bacteria. That this is due to the initiative of the leucocytes after the blood has been ingested, would seem to follow from the fact that they are massed within the largest and, presumably, oldest colonies, out of all proportion to the entire area infected; that is taking the smaller and scattered masses of bacilli into account as well as the large masses which are usually very few in comparison with the former.

Another interesting feature is the striking differences of development that generally obtain in the bug from that found in the flea. In the former, growth arises and tends to continue as isolated colonies, while in the flea the growth speedily loses its localized form and becomes general, often filling the entire stomach with a cohesive mass. It seems probable that this divergence is due to the speedy breaking down of the blood which takes place in the stomach of the flea, while in the bug the cellular structure of the blood would appear to be present for days as against a fraction of an hour or so in the flea. As regards the various phases of development exhibited by different bugs, this may be in large measure due to the volitional character of the bug's digestive apparatus. Verjbitzki called attention to the fact that starved bugs gave a different reaction to the more recently fed specimens; the implication being that the starved insects were the more thrifty in the use of the blood that they had ingested.

The general appearance of the specimens suggests that growth is at first rapid, but afterwards slows down and loses its vigour as it spreads outwards, leaving an exhausted area within the growing fringe, much as the small toadstools which form "fairy rings" in the turf are stated to do.

There are curious divergences in the development of the growth of colonies even in specimens of the same age which were fed on the same animal, kept under identical conditions for the same period and then fixed and sectioned under at one and the same time. Such differences must seemingly rest with the bug itself and are probably due to variations in the digestive action of the different specimens.

Cases of infection following the bites of bugs carrying B. pestis.

Only on two occasions did death follow from the bite of infected bugs and there was one doubtful case of infection when a rat fell sick and showed some of the symptoms of an animal suffering from pest, but it subsequently recovered.

A number of trials were made with two separate batches of infected bugs each consisting of larvae, nymphs and adults. The insects were kept in glass tubes and allowed to feed through a gauze covering on the shaved abdomen of the rat—the gauze cover being changed before each experiment. A number of rats were tried and the insects were in most cases allowed to feed on the same rat on several occasions. The result, as stated above, was one death, and one possibly infected rat which did not die, out of seven or eight rats.

The other experiment which gave a positive result was with a mouse. A batch of bugs, consisting of well-grown larvae, some nymphs and several adults were fed on a mouse just prior to its death from pest. They were then kept in a card-sided glass-bottomed box at about 60—65° F. without further opportunities of feeding. From this stock were drawn a number of the examples already discussed as smears or sections.

The survivors died off gradually until on the forty-eighth day there were only five living and one of these was very feeble. These five survivors were placed in a deep glass jar with some crumpled filter paper to afford them foothold and cover and a healthy mouse was put in. On the following morning only one of the bugs could be found, the others must have been eaten by the mouse, but the sole survivor had managed to feed.

Five days later the mouse died of typical pest. The glands of the right groin and axillae were both infected, while smears of the spleen and heart blood also contained numbers of *B. pestis*. Mice inoculated with the organisms recovered from the liver of this specimen also succumbed to the disease. Although a number of attempts were made to feed the surviving bug on mice and the ear of a guinea-pig, it could not be induced to bite and was killed when very feeble 74 days after its infected meal[1].

Discussion of the possible mechanism of infection by bugs.

The marked difference in the development of *B. pestis* in fleas and bugs is probably due to the wide divergence in their structure and habits. The digestive processes of the flea result in a rapid destruction of both red blood cells and leucocytes, leaving the contents of its stomach very much in the condition of an artificial culture media, if we may judge from the development of the bacilli. In the bug, on the other hand, the crop would seem to serve chiefly as a reservoir for the storage of food: the blood within it remaining, so far as structural appearances go, unaltered for days after its ingestion. Sections show this organ to act mainly as a simple walled sack. At its posterior end only does the development of the epithelial cells suggest that any digestive process is possible. Whether as a consequence or as an accompanying phenomenon, the conditions within the crop of the bug do not favour the same comprehensive development of *B. pestis*, which is normal in the flea.

[1] See note under "Sections" of the last specimen which was killed and cut 74 days after the infecting meal.

Behind the crop in the long tubular portion of the stomach which continues back to the malpighian tubes, digestion is very evident, and in this area occasionally a more comprehensive growth takes place. As a rule, however, the digestive process seems powerful enough and sufficiently rapid to prevent any large area being occupied by an evenly staining growth of *B. pestis*.

As regards structure there is a very marked difference between fleas and bugs as to the junction of the oesophagus and stomach. Bacot and Martin (1914) show that the blocking of the intricate valvular proventriculus of the flea is a crucial factor in the mouth transmission of plague by these insects. With the bug the likelihood of any such stoppage is improbable. The large flat head of the *Cimex lectularius* accommodates a much more powerful pump than does the narrow laterally compressed head of the flea. It follows that the relative ratio of the pump to oesophagus in cross section is very different in the two insects, the dimensions of pumping chamber in the bug being very many times that of the short tubular oesophagus through which the blood passes into the crop. In conjunction with the alimentary system of the flea that of the bug might be described as valveless. Actually the oesophagus, as seen in sections, is found to be thick-walled with a folded and puckered inner lining which greatly obstructs the lumen of the tube. The appearance premises great capabilities of distension or extension under stress of circumstances, but little in the way of effective control of any inward or outward flow of blood, while I have been unable to trace any muscles or muscular attachments which appear capable of any constrictive power. The action of the crop itself, however, as it fills and distends in all directions will not only allow of the shortening of the oesophagus, but may finally, as it pushes forwards, cause the oesophagus to take up a more or less transverse or even a folded position which may be sufficiently acute to close the tube. Although the arrangement appears to be slipshod in the last degree, the pressure of the blood within the crop possibly proves effectual enough in preventing egress of the contents of the crop in practice. If it is less perfect from a mechanical point of view than the valve of the flea, there is little danger of any growth of *B. pestis* being able to effect a stoppage.

It has already been noted in the descriptions of the sections that *B. pestis* does actually multiply among the folds of the lining membrane of the oesophagus, and in one specimen small groups were to be seen in the act of passing out of the narrow entrance into the pumping chamber. The chances of any direct regurgitation of infected blood from the crop

into the wound made by the bug when feeding are lessened owing to the disparity in size between the oesophagus and the pumping chamber, as it is necessary that the latter be filled before any regurgitation can take place.

We must, however, bear in mind the fact that when a bug is disturbed during its meal that it will feed again within a short interval, and it seems not impossible that injected blood might at the moment of interruption be regurgitated through the pump and washed down into the wound at the next attempt by the flow of saliva which enters into the pharynx anterior to the pump. If such regurgitation takes place the loose character of the colonies within the crop in conjunction with the efficient mixing apparatus afforded by a powerful pump jerking fluid through a narrow tube into an elastic bag would seem to be an ideal aid to the chances of a sample of any bacteria present in the crop being mixed with the regurgitated fluid.

This explanation is, of course, mere speculation, but there seems to be no doubt that the infection which followed Verjbitzky's experiments was due to regurgitation.

In the cases I have recorded above one may possibly have arisen owing to injury to a bug by the mouse while it was feeding, but in the case of the rat it must have been due to simple regurgitation.

Rectal infection may, I think, be safely dismissed, owing to the fact that bugs, as pointed out by Martin (1913), do not defaecate during meals, as fleas are occasionally known to do, but hurry after their meal to some nook or cranny to digest at leisure.

Conclusions.

(1) That for a percentage of bugs (*Cimex lectularius*) and probably all newly hatched ones, a meal of septicaemic blood from a mouse dying of plague is fatal.

(2) Bugs which are not killed by the infecting meal are capable of carrying *B. pestis* and reinfecting mice after a period of 48 days' starvation.

(3) The development of *B. pestis* within the crop of bugs differs generally from that which takes place in the stomach of the flea in respect of its slower and looser growth, this limitation of activity being accompanied by and possibly due to the preservation of the structural character of the blood for many days after its ingestion into the crop.

(4) The absence of any definite valve between the pump and the crop, together with the looser nature of the growth within the bug, preclude the idea of such mechanical blockage as causes regurgitation and mouth infection by fleas. It may be surmised, however, that mouth infection when not caused by accidental or other injury to the bug while feeding, may be due to interruption followed by a second attempt.

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EXPLANATION OF PLATES.

Plate XXXVII. Fig. 1. Transverse section through middle of crop of bug (*Cimex lectularius*) in first instar. Plague infection. 1 day at 60—65° F. *a*. Wall of crop. *b*. Infected blood. *c*. Uninfected blood. *d*. Phagocytes. (×180.)

Fig. 2. Portion of Plate XXXVII, fig. 1. *a*. Wall of crop. *b*. Blood infected with plague bacilli. *c*. Uninfected blood. *d*. Phagocytes. (×1000.)

Fig. 3. Portion of Plate XXXVIII. *a*. Autolyzed material containing bacilli. *b*. Phagocytes. *c*. Uninfected blood. (×1000.)

Plate XXXVIII. Longitudinal vertical section of adult bug (*Cimex lectularius*). Plague infection. 2 days at 60—65° F. *a*. Integument. *b*. Pharyngeal muscles. *c*. Pharyngeal pump. *d*. Oesophagus filled with blood. *e*. Crop. *f*. Ant. cerebral ganglion. *g*. Post. cerebral ganglion. *h*. Ventral nerve cord. *i*. Phagocytes in autolyzed blood. (×135.)

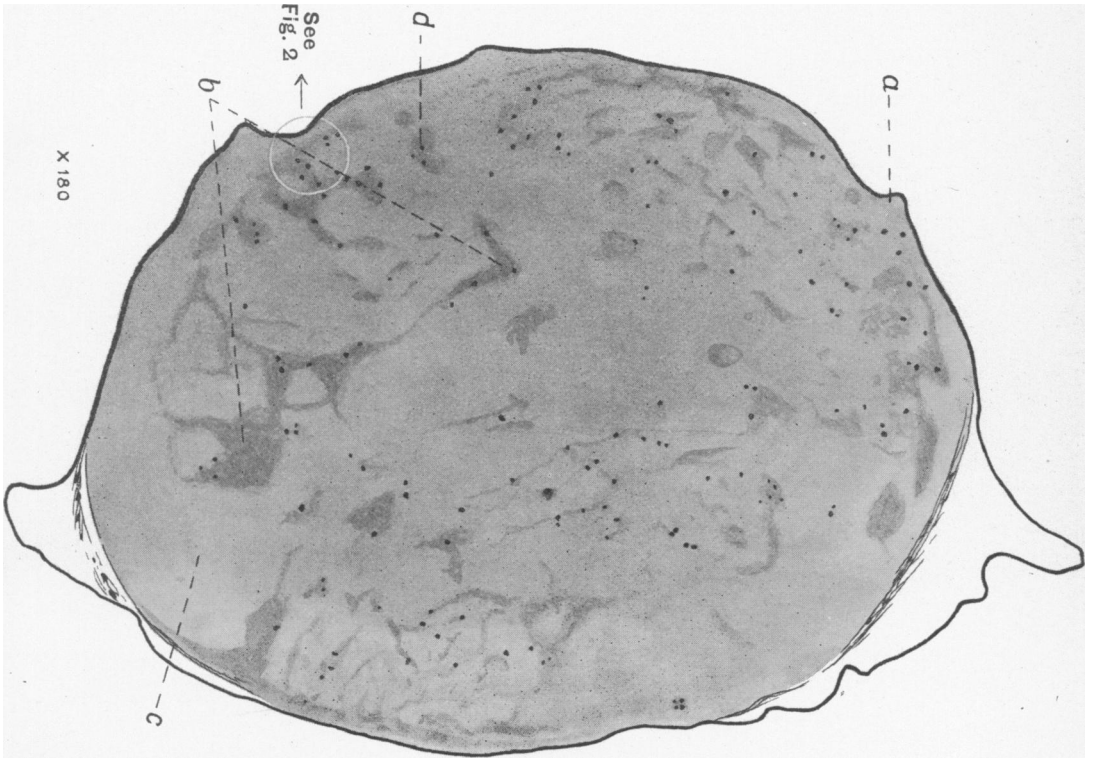

Fig. 1.

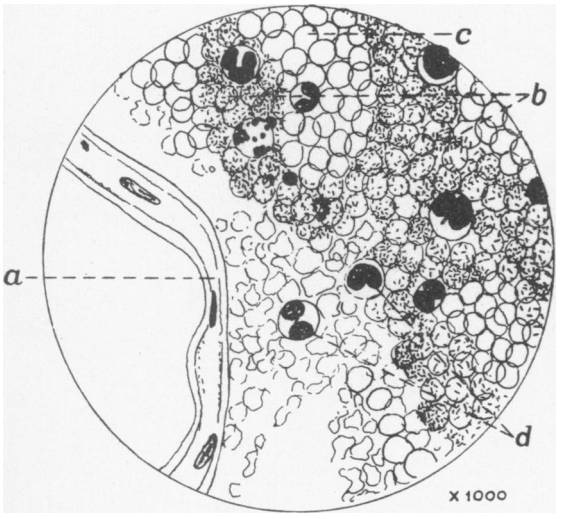

Fig. 2.

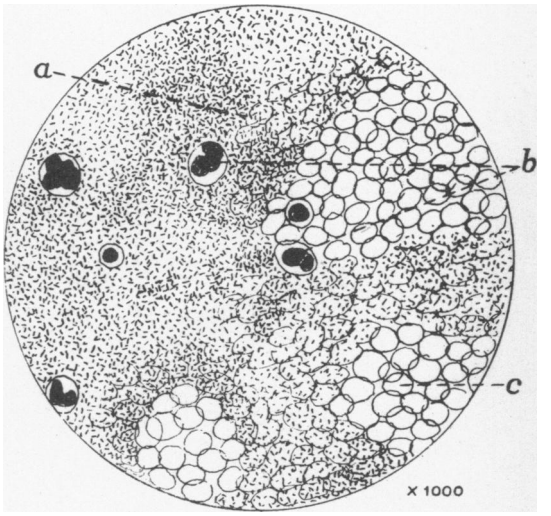

Fig. 3.

Mabel Rhodes del.

See Plate XXXVII Fig. 3
a
b
c
d
e
f
g
h
i
X 135
Mabel Rhodes del.